Oxidative stress is associated with C-reactive protein in non-diabetic postmenopausal women, independent of obesity and insulin resistance

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ABSTRACT

Oxidative stress is associated with C-reactive protein in non-diabetic postmenopausal women, independent of obesity and insulin resistance

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Objective: Oxidative stress is associated with obesity, metabolic syndrome, and inflammation, suggesting it could be an early event in the pathology of chronic diseases. We tested the hypothesis that elevated levels of oxidative stress markers are associated with increased C-reactive protein (CRP) and that this is independent of obesity and insulin resistance.

Research design and methods: This study was cross-sectional designed and non-diabetic postmenopausal women (n=1821) with CRP levels ≤10 mg/L was enrolled.

The CRP levels were categorized into quartiles from the lowest to the highest concentrations (Q1-Q4). The degree of insulin resistance was determined using the homeostasis model assessment of insulin resistance (HOMA-IR). We measured oxidative stress using urinary 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) and plasma oxidized low-density lipoprotein (ox-LDL).

Results: After adjustments for age and lifestyle habits, including smoking and drinking, we found higher body mass index (BMI) and HOMA-IR scores in Q2 and Q3 versus Q1. The Q4 BMI and HOMA-IR scores were higher than all other quartiles. The plasma ox-LDL was higher in Q4 than in Q1. Urinary 8-epi-$PGF_{2\alpha}$ was higher in Q3 and Q4 than in Q1 or Q2. Urinary 8-epi-$PGF_{2\alpha}$ positively correlated with CRP ($r=0.235$, $P<0.001$) whereas no correlation was found between ox-LDL and CRP. Multiple linear regression analyses of BMI and HOMA-IR showed that the association between urinary 8-epi-$PGF_{2\alpha}$ and CRP levels remained significant ($P<0.001$).

Conclusions: Oxidative stress measured by increased concentration of urine 8-epi-$PGF_{2\alpha}$ is strongly associated with CRP levels. This finding was independent of obesity and insulin resistance in non-diabetic postmenopausal women.

Keywords : oxidative stress; menopause; C-reactive protein; oxidized LDL(ox LDL); Isoprotanes (8-epi-$PGF_{2\alpha}$)

1. INTRODUCTION

When the production of reactive oxygen species increases, the balance between oxidant and antioxidant factors is disturbed and a pro-oxidative condition results. (1) This oxidative stress can damage cellular structures and trigger an inflammatory cascade, which induces more free radical production and causes a vicious positive feedback loop (2-4). Oxidative stress has been associated with obesity and metabolic syndrome (5,6), as well as inflammation, suggesting that oxidative stress may be an early event in the pathology of chronic diseases, including diabetes and cardiovascular disease (CVD) (7). Also, it has been suggested that oxidative stress could be induced by low-grade systemic inflammation (8), mainly characterized by higher concentrations of CRP and interleukin-6 (9). A low-grade inflammatory state that induces the production of free radicals and leads to increased lipid peroxidation (10). Moreover, inflammation acts as a preceding event to carcinogenesis. Inflammation-induced generation of reactive oxygen species (ROS) causes oxidative damage of target cell DNA, thereby initiating cellular transformation and oxidative stress-induced inflammation can also promote carcinogenesis (11).

Menopause is the permanent cessation of menstruation owing to loss of ovarian follicular function that produces an abrupt drop in estrogens and, therefore, the classic signs and symptoms and increased risk of cardiovascular diseases and osteoporosis (12). The postmenopausal period may be considered the beginning of the aging process in women, which is caused by a series of endocrinological changes caused by the decline of estrogen levels. Once women begin ovarian senescence, estrogen

production becomes erratic, antioxidant protection is lost, and oxidative stress is assumed to increase. Thus, it is possible that menopause is a risk factor for oxidative stress. As a matter of fact, the lipoperoxide levels were significantly higher in the postmenopausal group than in the premenopausal group (13).

The postmenopausal women, a risk population of oxidative stress, this condition influence the association between postmenopausal state and comorbidities. High levels of urinary 8-epi-$PGF_{2\alpha}$ were associated with increased incidence of fatal cardiovascular disease (CVD), which is including coronary heart disease (CHD). Women who were in the highest quartile of 8-epi-$PGF_{2\alpha}$ levels had, independently of age, an odd ratio of 1.8 for CVD mortality (14). In this study, we measured the urinary concentration of two such markers, creatinine-indexed 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) and plasma oxidized low-density lipoprotein (ox-LDL), in non-diabetic postmenopausal women (n=1821). We tested whether elevated levels of oxidative stress markers are associated with increased C-reactive protein (CRP) in non-diabetic individuals. Further, we evaluated if these relationships are independent of obesity and insulin resistance.

2. BACKGROUND

2.1. Menopause

Natural menopause is defined as the permanent cessation of mensturation resulting from the loss of ovarian follicular activity that leads to an abrupt drop in estrogens. (15, 16)

This estrogen is known to play a wide variety of roles. Estrogen protects renal by inhibiting component of RAAS (renin-angiotensin-aldosterone system), including angiotensin type 1 receptor expression, and by reducing angiotensin-converting enzyme activity. And endogenous 17β-estradiol has vasodilator activities. (17) Also an antioxidant effect of 17β-estradiol is reported that administration of 17β-estradiol significantly inhibited the oxidation of LDL from postmenopausal women. (18) Therefore this estrogen decline play important role in the cause of many symptoms common in middle age and may contribute to neurovegetative and urogenital disturbances along with chronic conditions such as postmenopausal osteoporosis and cardiovascular disease(CVD). (19)

2.2. Inflammation marker

2.2.1. Oxidized LDL and atherosclerosis.

An elevated plasma level of low-density lipoprotein (LDL) is clearly a major risk factor for cardiovascular disease. Several lines of evidence are reported oxidative modification of LDL as an atherogenic agent. (20)

The initial process of atherosclerosis is started endothelial injury, which leads to oxidation and accumulation of LDL in subendothelial space of vessel. (21) Oxidized LDL promotes the chemotaxis of monocyte into the vessel wall by modulating the secretion of numerous cytokines, chemokines and adhesion molecules.

Intercellular adhesion molecule-1(ICAM-1) enhances the adhesion of monocytes, neutrophils and lymphocyte. Endothelium leukocyte adhesion molecule (ELAM-1) stimulates monocyte and T-lymphocyte interaction. And vascular adhesion molecule (VACM-1) results in binding of monocyte and T-lymphocyte. (22) Thereafter trapped monocytes differentiate into macrophages that secrete specific chemoattractants (MCP-1) for monocytes. And marchrophages take up oxidized LDL through scavenger receptors (SCs), resulting in foam cell generation. (23) Oxidized LDL stimulates both the migration of smooth muscle cells (SMCs) from media into intima via induction of platelet-derived growth factors (PDGF) and proliferation via induction of basic fibroblast growth factor (bFGF) in endothelial cells. (Fig.1.) Accumulation of foam cells and SMCs leads to fibrous cap formation resulting in

reduction of diameter of the lumen of the artery, which can further evolve to plaque rupture and thrombosis.

Oxidized LDL has been reported to down regulate of nitric oxide (NO) production and endothelial relaxing factor (EDRF). (21) Inactivation of NO is another factor of atherosclerosis. Nitric oxide is powerful endogenous vasoprotectant that has anti-inflammatory, anti-aggregatory, anti-oxidant and vasodilator properties. Inhibition of NO production and activity has been implicated in the development of atherosclerosis. (22)

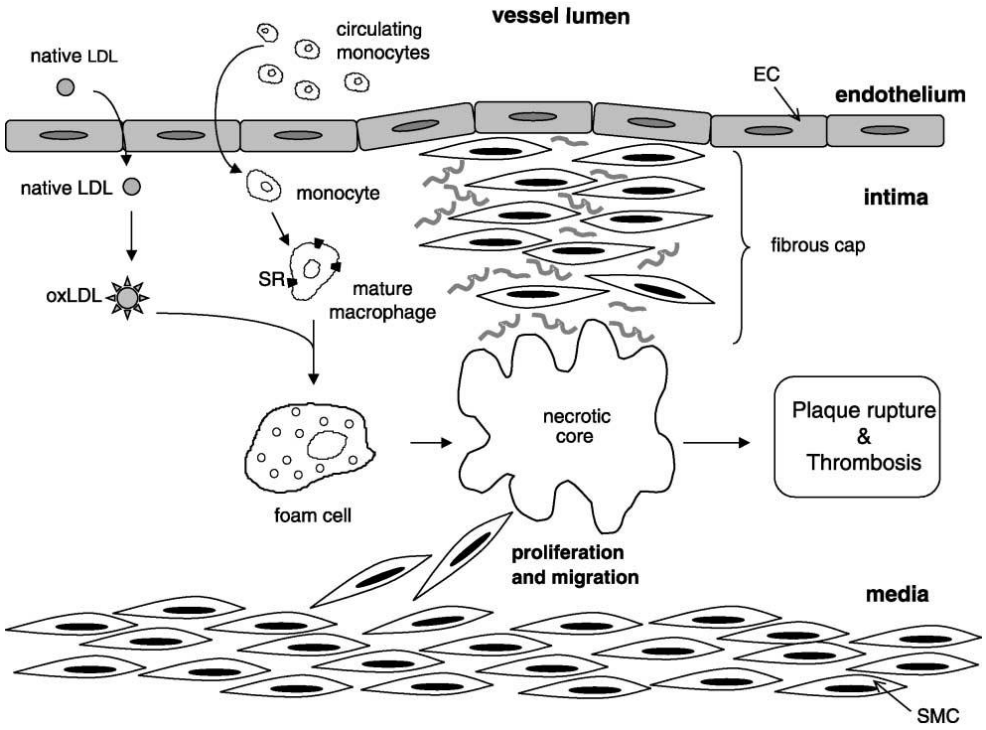

Figure 1. Description of the atherosclerotic process.(adopted from (23))

2.2.2. The characteristics of $PGF_{2\alpha}$

Free radical has been implicated in the pathophysiology of a wide variety of human disorders (24). One of the well-recognized targets of free radical-induced injury is lipid peroxidation. (25) Isoprostanes, a group of prostaglandin-like compounds, are biosynthesized primarily from esterified arachidonic acid by a non-enzymatic free radical-catalyzed reaction in vivo. Several denominations of F_2-isoprostanes have been used in the literature such as 8-iso-$PGF_{2\alpha}$, 8-epi-$PGF_{2\alpha}$, F_2-isoPs, 15-F_2-isoPs, 15-F_{2t}-isoPs, iPF2alpha-Ⅲ, iPF2alpha-Ⅵ. (26) 8-epi-$PGF_{2\alpha}$ is a major component of the F_2-isoprostanes family which has been found to modulate platelet aggregation. And 8-epi-$PGF_{2\alpha}$ is a potent renal and pulmonary vasocontrictor in rat kidney and lungs. (27, 28) Elevated isoprostane levels are associated with severe acute and chronic inflammatory diseases such as asthma, atherosclerosis, chronic obstructive pulmonary diseases (COPD), ischemia-reperfusion, rheumatic disease, septic shock, diabetes, ect. (29) F2-isoprstanes esterified to tissue or plasma lipids or in the free form in body fluids have in fact proved to be useful indicators of local or systemic oxidant stress (30)

Isoprostanes from arachidonic acid precursor is formed through the many processes. An abstraction of a bisallylic labile hydrogen atom occurs first, and addition of an oxygen molecule to arachidonic acid leads to four forms of positional peroxyl radicals. Further, endocyclization occurs, and an oxygen molecule is added to form four unstable PGG_2-like bicyclic endoperoxide intermediate. And then reductions by the

presence of glutathione result in the parent isopostanes of various series. (26) Four F-ring isoprostane regioisomers are named as 5-, 8-, 12-, or 15 series regioisomers on the basis of carbon atom to which the side chain hydroxyl-group is positioned. (Fig.2. (31)) And several other isoprostanes of series D2, and E2, thromboxane A2, cyclopentanone-A2 and -J2 are also formed in vivo by rearrangement of PGH-2 like isoprostane intermediate.

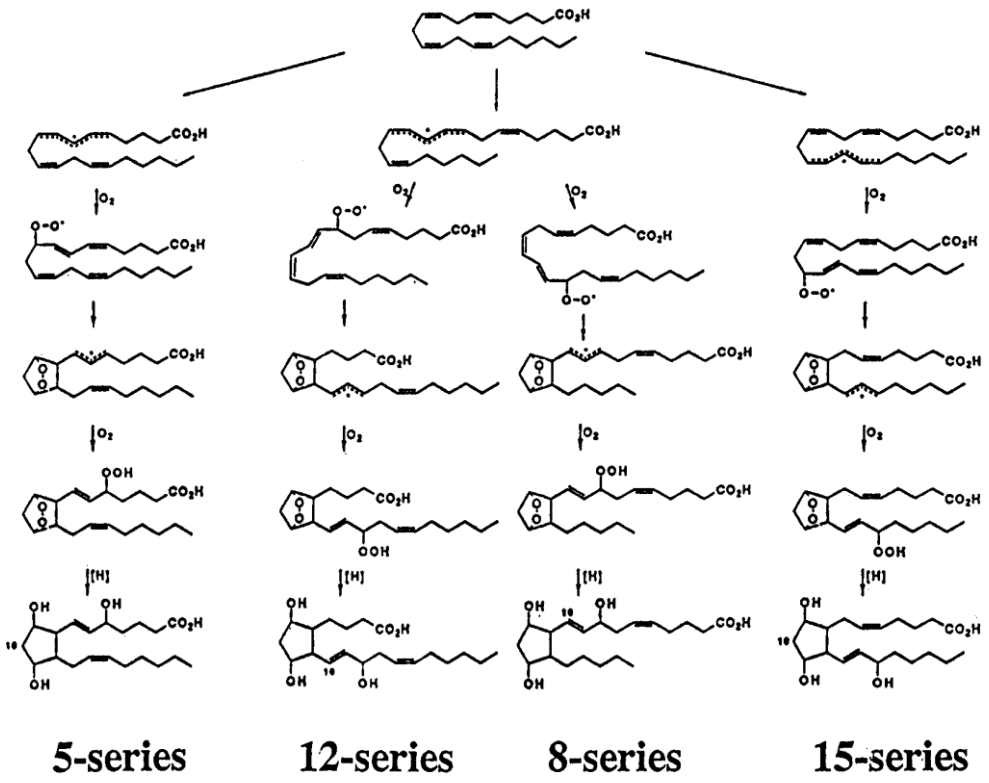

Figure 2. Mechanism of formation of isoprostanes.(adapted from (31))

A simplified mechanism of formation of isoprostanes and prostaglandins from arachidonic acid precursor is shown in Fig.3. This figure indicates conceivable relationships between inflammatory response and oxidative injury through enzymatic and non-enzymatic oxidation of arachidonic acid. (32)

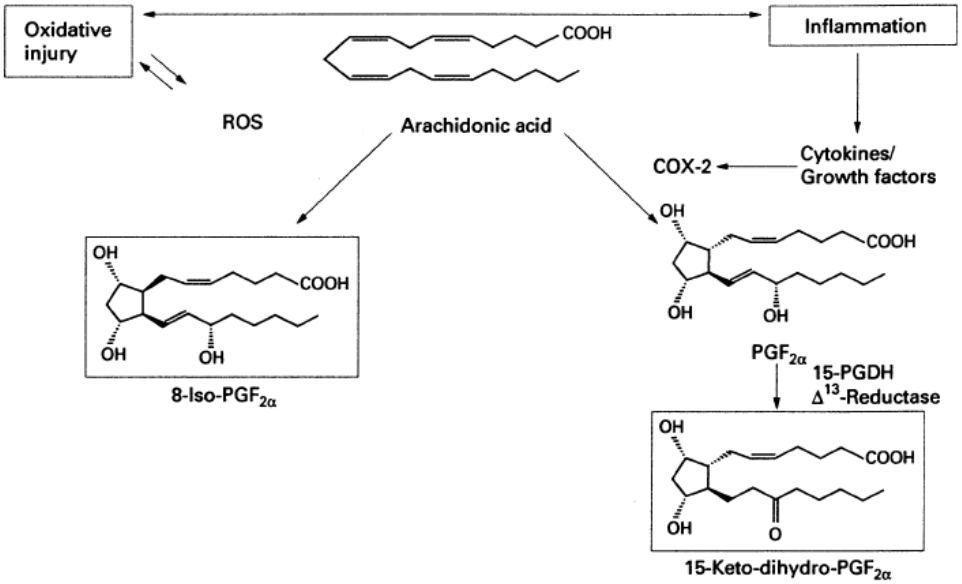

Figure 3. Relationship between non-enzymatical free radical and Cox catalyzed reactive pathways. ROS: reactive oxygen species, COX: cyclooxygenase, 15-PGDH: 15-hydroxy prostaglandin dehydrogenase, D13 Reductase : delta-13 reductase.(adapted from (32))

2.3. C-reactive protein (CRP)

With the recognition that atherosclerosis is an inflammatory process, several inflammation markers have also been evaluated as potential tools for prediction of the cardiovascular disease.(33) C-reactive protein(CRP) is a positive acute phase protein synthesized by the liver in response to inflammation and infection(34). Indeed CRP is thought to represent a state of chronic low-grade inflammation of the aterial vessel wall at atherosclerotic sites. Consistently CRP is a marker of cardiovascular outcome. (35)

CRP elicits a multitude of effects on endothelial biology that might promote atherosclerotic processes and endothelial cell (EC) inflammation. CRP downregulates endothelial NO synthase (eNOS) transcription and destabilize eNOS mRNA. Concurrently, CRP stimulates ET-1, MCP-1 and IL-6 release. And it induces expression of the adhesion molecule and facilitates EC apoptosis. CRP also potently upregulates nuclear factor κB, a important factor stimulating transcription of numerous proatherosclerotic genes. (Fig.4.) (36)

By binding to degraded low-density lipoprotein, CRP is able to activate complement via the classical pathway serving as a potential link between complement activation and atherosclerosis. (37) Also by upregulating the expression of complement inhibitor proteins in endothelial cells, CRP may protect ECs from complement-mediate cell lysis. A balance of proatherogenic and antiatherogenic effect of CRP may be important in development of atherosclerosis. (38)

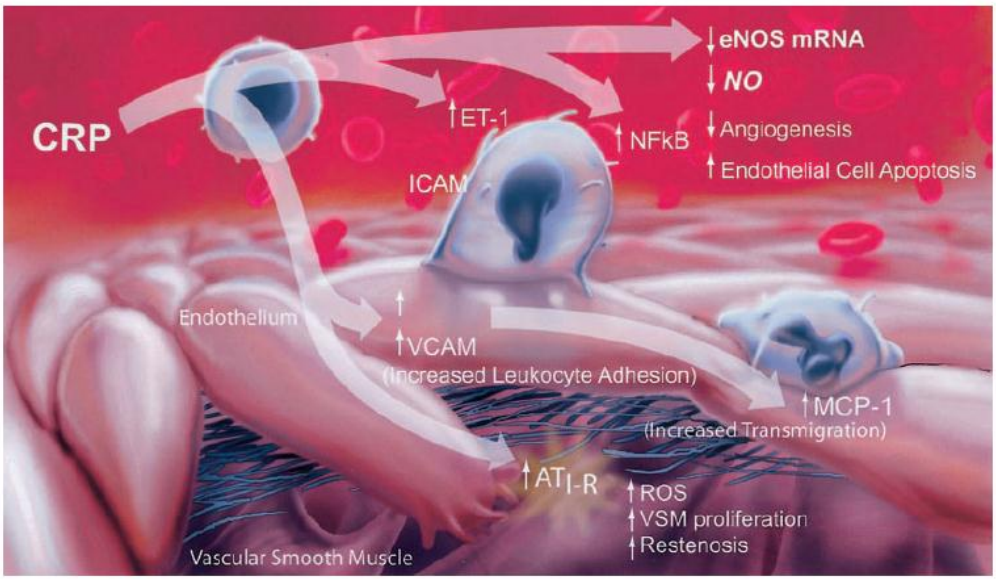

Figure 4. Function of C-reactive protein as an important marker of endothelial disfuntion.(adapted from (36))

3. METHODS

3.1. Participants and study design

A total of 1821 postmenopausal women who visited a National Health Insurance Corporation of Ilsan Hospital Health Promotion Center (Korea) between March 2009 and June 2011 were enrolled in this study. Exclusion criteria included a previous history of type 2 diabetes, CVD, cancer, abnormal liver or renal function, thyroid or pituitary disease, and use of any medications (including anti-hypertensive, lipid lowering, anti-platelets, and anti-diabetic drugs). The purpose of the study was carefully explained to all participants and written consent was obtained prior to their participation. Participants also completed a personal health and medical history questionnaire, which served as a screening tool. The Institutional Review Board of the National Health Insurance Corporation of Ilsan Hospital approved the study protocol, which was conducted in accordance with the Declaration of Helsinki. Postmenopausal status was defined as an absence of menstruation for at least 12 months and the presence of estrogen deficiency symptoms including hot flashes, increased sweating, nervousness, irritability, depression, palpitations, insomnia, headaches, dyspareunia, and joint pain. The high sensitivity CRP (hs-CRP) levels were categorized into quartiles from the lowest to the highest concentrations (Q1-Q4). Q1 was defined as a hs-CRP level <0.3 mg/L, Q2 was 0.3-0.58 mg/L, Q3 was 0.58-1.24 mg/L, and Q4 was 1.24-10.0 mg/L.

3.2. Anthropometric parameters, blood pressure, and blood collection

Body weight and height of unclothed participants without shoes were measured in the morning to calculate body mass index (BMI; kg/m^2). Waist circumference was measured at the umbilical level with the participants standing after normal expiration. Blood pressure (BP) was measured on the left arm of seated patients with an automatic BP monitor (TM-2654, A&D, Tokyo, Japan) after 20 min rest. Study participants were questioned about their lifestyle habits, including smoking and drinking, during their visit. After a 12-hour fasting period, venous blood specimens were collected in EDTA-treated and plain tubes, centrifuged to produce plasma or serum, and stored at -70°C until analysis.

3.3. Serum lipid profile, white blood cell count, fasting glucose, insulin concentration, homeostasis model assessment-insulin resistance (HOMA-IR), and free fatty acid measurements

Fasting serum concentrations of total-cholesterol and triglycerides were measured using commercially available kits on a Hitachi 7150 Autoanalyzer (Hitachi Ltd., Tokyo, Japan). After precipitation of apoB-containing lipoproteins with dextran sulfate-magnesium, the supernatant concentration of high-density lipoprotein-cholesterol was measured enzymatically. LDL-cholesterol was indirectly estimated in participants with serum triglyceride concentrations less than 400 mg/dL using the Friedewald formula: LDL-cholesterol = total-cholesterol - [HDL-cholesterol +

(triglycerides/5)]. White blood cell (WBC) counts were determined using a hematology analyzer from HORIBA ABX Diagnostic (HORIBA ABX SAS, Parc Euromedicine, France). Fasting glucose levels were measured using a glucose oxidase method with a Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA). Insulin levels were measured by radioimmunoassay using commercial kits from Immuno Nucleo Corporation (Stillwater, MN). IR was calculated from the HOMA using the following equation: HOMA-IR = [fasting insulin (μIU/mL) × fasting glucose (mmol/L)] /22.5. Free fatty acids were analyzed with a Hitachi 7150 Autoanalyzer (Hitachi Ltd, Tokyo, Japan).

3.4. Plasma ox-LDL, urinary 8-epi-$PGF_{2\alpha}$, and serum hs-CRP measurements

Plasma ox-LDL was measured using an enzyme immunoassay (Mercodia, Uppsala, Sweden). The resulting colorimetric reaction was measured at 450 nm on a Wallac Victor2 multi-label counter (Perkin Elmer Life Sciences, Turku, Finland). After a 12-h fast, urine was collected in polyethylene bottles containing 1% butylated hydroxytoluene. The bottles were immediately covered with aluminum foil and stored at -70℃ until further analysis. The compound 8-epi-$PGF_{2\alpha}$ was measured using an enzyme immunoassay (BIOXYTECH urinary 8-epi-$PGF_{2\alpha}$™ Assay kit, OXIS International Inc., Portland, OR). Urinary creatinine levels were determined using the alkaline picrate (Jaffe) reaction. Inter and intra-assay precision was 13.8% and 5.4%. Serum hs-CRP concentrations were measured using an Express Plus™ auto-analyzer (Chiron Diagnostics Co., Walpole, MA, USA) with a commercially available, high-

sensitivity CRP-Latex(II) X2 kit (Seiken Laboratories Ltd., Tokyo, Japan). Inter and intra-assay precision was 2.1% and 1.5%.

3.5. Data analyses

Statistical analyses were performed using SPSS version 12.0 for Windows (SPSS Inc., Chicago, IL, USA). Skewed variables were logarithmically transformed for statistical analyses. Frequency was tested with the chi-squared test. One-way ANOVA with the Bonferroni correction was used to test the effects of hs-CRP levels (Q1: <0.3mg/L, Q2: 0.3-0.58mg/L, Q3: 0.58-1.24mg/L, Q4: 1.24-10.0mg/L). General linear model analyses were also performed with adjustments for age and lifestyle habits, including smoking and drinking. Pearson and partial correlation coefficients were used to examine the relationships between variables. Multiple stepwise regression analyses were performed to identify major factors associated with increased oxidative stress, according to hs-CRP levels. For descriptive purposes, mean values are presented using untransformed values. Results are expressed as the mean ± standard error. A two-tailed P value of less than 0.05 was considered statistically significant.

4. RESULTS

4.1. Relationships between CRP and conventional cardiovascular risk factors

The means of the conventional cardiovascular risk factors according to the CRP quartiles (Q1-Q4), after adjustment for age and lifestyle habits, include alcohol consumption and cigarette smoking, are shown in Table 1. BMI, insulin, and HOMA-IR were higher in Q2 and Q3 than in Q1. The Q4 scores were higher than the Q1, Q2, and Q3 scores. Waist circumference was higher in Q3 and Q4 than in the other quartiles. Q2 and Q4 had higher systolic blood pressure than Q1 but Q2 displayed higher diastolic blood pressure than Q1. Triglycerides were higher in Q3 and Q4 than in Q1 or Q2, however, HDL-cholesterol was lower in Q3 than in Q1 and lower in Q4 than both Q1 and Q2. Q4 had higher total cholesterol than Q2 and higher LDL-cholesterol than Q2 and Q3. The participants in Q4 also had the highest glucose and leukocyte numbers.

4.2. Relationship between CRP and oxidative stress markers

Figure 5 shows the distribution of plasma ox-LDL and urinary 8-epi-$PGF_{2\alpha}$ after participants were classified according to CRP quartiles. Plasma ox-LDL was higher in Q4 than in Q1 after adjusting for age and lifestyle habits. Urinary 8-epi-$PGF_{2\alpha}$ was higher in Q2 and Q3 than in Q1 and Q4 values were higher than both Q1 and Q2.

Table 1. Age and lifestyle adjusted means of participants′ characteristics according to CRP quartiles

	Q1 (n=449)	Q2 (n=458)	Q3 (n=458)	Q4 (n=456)	*P*
CRP level (mg/L)	< 0.3	0.3 - 0.58	0.58 - 1.24	1.24 - 10.0	-
Body mass index (kg/m^2)	23.1 ± 0.12	23.8 ± 0.12*	24.1 ± 0.12*	24.8 ± 0.12*†‡	< 0.001
Waist (cm)	84.9 ± 0.36	85.9 ± 0.36	86.6 ± 0.36*	88.0 ± 0.36*†‡	< 0.001
Systolic BP (mmHg)	123.1 ± 0.71	126.4 ± 0.69*	125.2 ± 0.69	126.8 ± 0.70*	0.001
Diastolic BP (mmHg)	75.2 ± 0.47	77.6 ± 0.46*	76.3 ± 0.46	76.8 ± 0.46	0.004
Triglycerides (mg/dL)§	105.2 ± 3.14	112.8 ± 3.07	128.3 ± 3.08*†	139.4 ± 3.09*†	< 0.001
HDL-cholesterol (mg/dL)§	58.1 ± 0.70	56.2 ± 0.69	54.4 ± 0.69*	52.3 ± 0.69*†	< 0.001
Total-cholesterol (mg/dL)§	203.1 ± 1.69	199.9 ± 1.66	202.8 ± 1.66	206.6 ± 1.66†	0.038
LDL-cholesterol (mg/dL)§	124.0 ± 1.54	121.2 ± 1.51	123.2 ± 1.51	127.4 ± 1.53†‡	0.024
Glucose (mg/dL)§	86.2 ± 0.52	85.3 ± 0.51	86.4 ± 0.51	88.3 ± 0.51*†‡	0.001
Insulin (uU/mL)§	7.44 ± 0.23	7.98 ± 0.22*	8.38 ± 0.23*	9.06 ± 0.23*†‡	< 0.001
^{1}HOMA-IR§	1.61 ± 0.06	1.70 ± 0.06*	1.82 ± 0.06*	2.01 ± 0.06*†‡	< 0.001
White blood cells (×10^9/L)§	4.94 ± 0.06	4.98 ± 0.06	5.08 ± 0.06	5.63 ± 0.06*†‡	< 0.001

Mean ± S.E., *p<0.05 compared with the value in the Q1 tested by ANOVA (Bonferroni correction). †p<0.05 compared with the value in the Q2 tested by ANOVA (Bonferroni correction). ‡p<0.05 compared with the value in the Q3 tested by ANOVA (Bonferroni correction). §measured by logarithmic transformation, *P*-values derived from ANOVA with Bonferroni correction. ^{1}HOMA-IR = {fasting insulin (μU/mL) x fasting glucose (mmol/L)}/22.5.

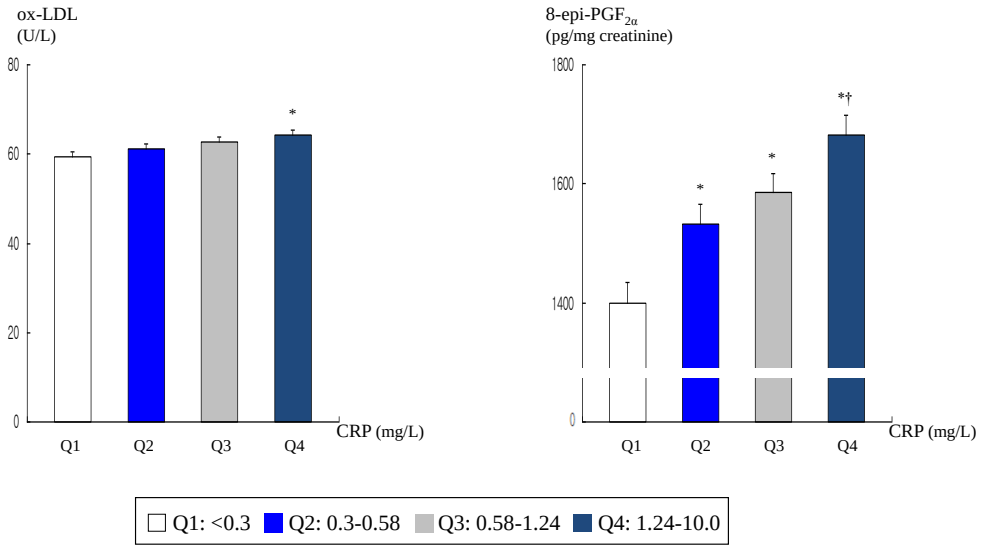

Figure 5.Comparison of plasma ox-LDL and urinary 8-epi-$PGF_{2\alpha}$ in 1821 non-diabetic postmenopausal women between the CRP quartiles (Q1-Q4) after adjusting for age and lifestyle. Mean ± SE, P-values derived from ANOVA with Bonferroni correction. $^{*}p<0.05$ compared with the value in the Q1 tested by ANOVA (Bonferroni correction). $^{\dagger}p<0.05$ compared with the value in the Q2 tested by ANOVA (Bonferroni correction).

4.3. Correlations between oxidative stress markers and metabolic syndrome factors

Table 2 displays the correlations between oxidative stress markers and factors of metabolic syndrome factors before and after age and lifestyle adjustments. Plasma ox-LDL levels positively correlated with BMI, diastolic blood pressure, triglycerides, total cholesterol, LDL-cholesterol, and HOMA-IR index. Urinary 8-epi-$PGF_{2\alpha}$ levels positively correlated with HDL-cholesterol and CRP. In addition, there was a weak negative correlation of urinary 8-epi-$PGF_{2\alpha}$ with LDL-cholesterol.

Table 2. Correlations between ox-LDL and 8-epi-$PGF_{2\alpha}$, and metabolic syndrome factors

	Oxidized LDL				**8-epi-$PGF_{2\alpha}$**			
	r_0	p_0	r_1	p_1	r_0	p_0	r_1	p_1
Body mass index	0.075	0.005	0.059	0.025	-0.026	0.317	-0.031	0.226
Systolic BP	0.108	<0.001	0.050	0.058	0.020	0.453	-0.012	0.656
Diastolic BP	0.071	0.008	0.060	0.023	-0.006	0.819	-0.011	0.686
Triglycerides[§]	0.235	<0.001	0.219	<0.001	-0.035	0.177	-0.048	0.063
Total-cholesterol[§]	0.359	<0.01	0.395	<0.001	-0.043	0.097	-0.035	0.178
HDL-cholesterol[§]	-0.056	0.034	-0.051	0.053	0.097	0.001	0.104	<0.001
LDL-cholesterol[§]	0.358	<0.001	0.398	<0.001	-0.078	0.003	-0.068	0.009
HOMA-IR[§]	-0.043	0.106	-0.052	0.049	0.030	0.249	0.034	0.188
hs-CRP[§]	0.080	0.003	0.015	0.582	0.248	<0.001	0.235	<0.001

r_0: Unadjusted. r_1: Correlation coefficient after adjusted for age and lifestyle. [§]measured by logarithmic transformation.

4.4. CRP in relation to oxidative stress, BMI and HOMA-IR

To evaluate the strength of the association between CRP and oxidative stress, as well as BMI, HOMA-IR and each metabolic syndrome component, multiple linear regression analyses were performed (Table 3). After adjusting for age and lifestyle habits, CRP significantly associated with urinary 8-epi-$PGF_{2\alpha}$, triglycerides, HDL-cholesterol, and systolic blood pressure. There was no significant association of CRP with ox-LDL or diastolic blood pressure. These variables were then adjusted with BMI and HOMA-IR, in addition to age and lifestyle. The association of urinary 8-epi-$PGF_{2\alpha}$ with CRP slightly increased after adjusting for BMI and HOMA-IR but the association of CRP with triglycerides and HDL-cholesterol was attenuated.

Table 3. Multiple linear regression analyses of the relationships between CRP, oxidative stress markers, and metabolic syndrome factors

	P1		***P2***	
	β coefficient (95%CI) [a]	*P* value	β coefficient (95%CI) [a]	*P* value
8-epi-$PGF_{2\alpha}$	0.487 (0.329, 0.646)	<0.001	0.504 (0.351, 0.658)	<0.001
Oxidized LDL	0.161 (-0.016, 0.338)	0.074	0.135 (-0.037, 0.307)	0.124
Triglycerides	0.389 (0.259, 0.519)	<0.001	0.224 (0.090, 0.358)	0.001
HDL-cholesterol	- 0.592 (-0.827, -0.356)	<0.001	- 0.372 (-0.607, -0.137)	0.002
Systolic BP	0.005 (0.001, 0.009)	0.025	0.000 (-0.004, 0.004)	0.968
Diastolic BP	0.005 (-0.002, 0.011)	0.151	- 0.002 (-0.009, 0.004)	0.479

[a] Confidence interval. *P1*: Adjusted for age and lifestyle. *P2*: Adjusted for age, lifestyle, BMI, and HOMA-IR. Calculation of triglycerides, HDL-cholesterol, HOMA-IR, oxidized LDL, and 8-epi-$PGF_{2\alpha}$ were based on log-transformed values.

5. DISCUSSION

In this cross-sectional study correlating oxidative stress markers and CRP concentration in non-diabetic postmenopausal women, we found a positive association between urinary 8-epi-$PGF_{2\alpha}$ and CRP. However, participants with higher CRP levels also had higher blood pressure, increased anthropometric, lipid, glucose, and leukocyte measurements, as well as a higher HOMA-IR index. These results confirm previous reports that CRP concentration is influenced by many clinical characteristics, metabolic syndrome factors, and HOMA-IR index (39-41), which also affect oxidative stress levels. To more precisely define the relationship between oxidative stress and CRP concentration, we examined this relationship in multiple linear regression models after adjusting for BMI and HOMA-IR, in addition to age and lifestyle. A positive association of urinary 8-epi-$PGF_{2\alpha}$ with CRP concentration was still evident after adjusting for BMI and HOMA-IR, indicating this association is independent of obesity and insulin resistance in non-diabetic postmenopausal women.

Urinary 8-epi-$PGF_{2\alpha}$, a reliable marker of oxidative stress, is a major F_2-isoprostane and is formed during lipid peroxidation (41). This molecule exerts pathophysiological effects, such as vasoconstriction (43), and has been proposed as an independent cardiovascular risk factor (44,45). The urinary F_2-isoprostane level is favorable as an epidemiological marker due to its chemical stability, independence of dietary lipid content, and low variability in individuals (46-49). The positive association between urinary 8-epi-$PGF_{2\alpha}$ and CRP in the 1821 non-diabetic postmenopausal women from this study disagrees with a previous finding describing no relationship between 8-iso-

$PGF_{2\alpha}$ and CRP in 289 healthy men (50). Additionally, Couillard et al. (51) found no correlation between 8-iso-$PGF_{2\alpha}$ and CRP in men with abdominal obesity. However, a recent report described a connection between low-level inflammation and oxidative status in 60 individuals considered high risk for CVD, including diabetes, history of stroke, angina, or cancer (52). Schwedhelm et al. (53) also found a positive correlation between 8-iso-$PGF_{2\alpha}$ and CRP in 93 CVD patients and 93 age- and sex-matched controls. These conflicting data may be attributed to differences in the patient characteristics, including sex, disease, or ethnicity.

F_2-isoprostanes levels have been used extensively as biomarkers of lipid peroxidation as a risk factor for atherosclerosis and other diseases (54); therefore, it is to expect a positive correlation between LDL-cholesterol and negative association with HDL-cholesterol. However, Table 2 showed inverse results. The evidence which supports this result is that HDL-cholesterol is the major lipoprotein carrier of plasma F_2-isoprostanes. Levels of F_2-isoprostanes were approximately twice as high in HDL-cholesterol as in LDL-cholesterol and were 50% higher than in VLDL-cholesterol (55). Additionally, the correlation coefficients were too weak and low, even though the *P* values were significant because of large sample size. Besides, LDL-cholesterol levels of Koreans are relatively lower than those of western societies (56), so that the correlation between 8-epi-$PGF_{2\alpha}$ and LDL-cholesterol was not shown positively compared to other recent studies.

Another parameter of lipid peroxidation, ox-LDL, failed to show a positive association with CRP in this study. A positive association between ox-LDL and CRP has been reported in men with abdominal obesity (51) or patients with acute coronary

syndrome (57). However, no correlation of ox-LDL and CRP was found in patients with type 2 diabetes (58) or healthy men (50). Furthermore, similar to our current study, no relationship between oxLDL and 8-iso-$PGF_{2\alpha}$ was reported in healthy men (50). These contradictory results might be attributed, at least in part, to circulating ox-LDL derivatives from ruptured atherosclerotic plaques. Indeed, increased ox-LDL levels showed a positive correlation with acute coronary event severity (57,59). There is substantial evidence that ox-LDL is present *in vivo* in atherosclerotic blood vessels (60,61). Because we only included non-diabetic postmenopausal women in this study, this could explain the small difference in plasma ox-LDL levels according to the CRP quartiles. These data indicate that evaluation of urinary 8-epi-$PGF_{2\alpha}$ could be superior to ox-LDL measurements in determining oxidative stress in non-diabetic postmenopausal women.

The strengths of this study include a large samples size and precise biomarker assays. However, there are several limitations of our study. First, because of the study's cross-sectional design, our results do not provide information on a causal relationship. Furthermore, we used the HOMA-IR index rather than a more precise measurement, such as the euglycemic-hyperinsulinemic clamp. However, studies indicate that HOMA-IR is a well-validated insulin resistance measurement in epidemiological studies and correlated well with reference techniques (0.58-0.88) (41). Despite these limitations, we have demonstrated that increased CRP concentrations, even if within the clinically normal range, strongly associated with 8-epi-$PGF_{2\alpha}$, a reliable oxidative stress marker. In addition, this association was independent of obesity and insulin resistance in non-diabetic postmenopausal women.

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국문요약

폐경 후 비당뇨 여성에서 산화스트레스와 C-반응성 단백질과의 상관관계

활성산소종이 증가하면, 산화요인과 항산화요인 사이의 불균형이 생기게 되면서 산화적 스트레스 상태를 만든다. 산화스트레스는 세포구조 손상과 염증단계를 유발하는데 이는 유리기의 생성을 유도하고 공격적인 양의 피드백 과정을 발생시킨다. 산화스트레스는 당뇨나 심혈관 질환(CVD)같은 만성질환의 발병 초기단계인 비만, 대사증후군, 염증과 관련이 있다. 또한 CRP, IL-6의 농도가 높은 것이 특징인 낮은 단계의 전신 염증은 산화스트레스를 일으킬 수 있으며 이는 자유기의 생성과 지질과산화를 증가시키고 암 발성의 전 단계로 작용한다. 활성 산소종 (ROS)의 염증생성은 세포 DNA의 산화적 손상을 일으키고, 세포변형을 일으켜 암 발생을 촉진할 수 있다.

폐경은 난포 기능의 손실로 인한 생리의 영구적 중지를 말하며, 갑작스런 에스트로겐의 감소는 폐경의 일반적 신호와 증상을 나타내게 하고 심혈관 질환과 골다공증의 위험률을 증가시킨다. 여성에게서 폐경 후 기간은 에스트로겐수준의 감소에 의한 내분비적 변화로 인한 것이며 노화의 시작이라고 생각할 수 있다. 여성의 난소가 노쇠하게 되면 에스트로겐 생산은 불규칙해지고 항산화 보호 기능을 잃게 되므로 산화스트레스가 증가할 것을 가정할 수 있다. 그러므로 폐경은 산화스트레스의 위험요소이며 실제연구에서도 폐경 후 그룹이 폐경 전 그룹보다 지질과산화 정도가 유의적으로 높은 것으로 나타났다. 폐경 후 여성은 산화스트레스의 위험 군으로서 다른 만성질환과의 관계에 영향을 줄 수 있다. 높은 수준의 소변내 8-epi-

$PGF_{2\alpha}$ 농도는 관동맥성 심장질환을 포함한 치명적인 심혈관 질환의 발생 증가와 관련 있다. 8-epi-$PGF_{2\alpha}$ 수준이 가장 높은 사분위수에 있는 여성은 나이에 독립적으로 심혈관 질환 사망률이 1.8배 높다고 한다.

본 연구는 상승된 산화스트레스 지표가 C-활성 단백질(CRP)의 증가와 관련이 있는지 알아보고자 하였으며 비만, 인슐린 저항성과 독립적으로 관련이 있는지 평가하고자 하였다. 단면적 연구로 디자인 되었으며 일산병원에서 2009 년에서 21011 년까지 CRP 수준이 10mg/L 이하인 폐경 후 여성 1821 을 모집하였다. 제 2 형당뇨, 심혈관 질환, 암, 간질환, 신장질환, 갑상선질환 및 전립선질환이 있는 환자와 항혈압제, 지질강하제, 항혈소판제, 항당뇨제와 같은 약물복용자는 대상자에서 제외되었다. 체질량 지수, 혈압, 허리둘레의 인체계측이 실시되었으며 12 시간 공복 후 혈액과 소변검사를 실시하여 혈청지질, 백혈구 수, 혈당, 인슐린농도, 자유지방산, 인슐린저항성 (HOMA-IR), hs-CRP 를 측정하였다. 본 연구에서는 산화스트레스 지표로서 혈장 ox-LDL 과 소변의 8-epi-$PGF_{2\alpha}$를 분석하였다. 대상자는 CRP 농도에 따라 사분위수로 분류되었으며 (Q1<0.3mg/L, Q2:0.3mg/L -0.58mg/L, Q3:0.58mg/L-1.24mg/L, Q41.24mg/L-10.0mg/L), 나이와 음주, 흡연 같은 생활습관을 조정하여 통계 분석하였다.

CRP 농도에 따른 심혈관 위험인자와의 관계성을 살펴본 결과, 체질량 지수와 HOMA-IR 은 Q1 그룹보다 Q2, Q3 그룹에서 수치가 높았으며, Q4 그룹이 다른 그룹보다 유의적으로 높은 수치를 보였다. CRP 와 산화스트레스 지표와의 관계를 살펴보았을 때 혈장 ox-LDL 은 Q1 그룹보다 Q4 그룹이 높았으며 소변 8-epi-$PGF_{2\alpha}$는 Q3, Q4 그룹이 Q1, Q2 그룹보다 높은 것을 확인 할 수 있었다.

8-epi-$PGF_{2\alpha}$는 CRP 와 유의적으로 관련이 있었으나 ($r=0.235$, $P<0.001$) ox-LDL 은 CRP 와 관련이 없음을 알 수 있었다. 다중회귀분석 결과 비만과 인슐린 저항성과는 독립적으로 CRP 가 소변 8-epi-$PGF_{2\alpha}$와 유의적인 상관성이 있었다.

본 연구는 많은 대상자 수와 정확한 생화학적 지표 분석을 장점으로 들 수 있으나 단면적 연구로 진행되었다는 점과 HOMA-IR 지수를 사용하여 인슐린 저항성을 측정하였다는 점을 한계점으로 들 수 있다. 하지만 이러한 한계점에도 본 연구는 CRP 의 수치가 높더라도 임상적으로 정상범위 안에 있을 때 산화스트레스 지표인 8-epi-$PGF_{2\alpha}$와 강한 관련이 있음을 증명하였다. 또한 이 결과는 비만과 인슐린 저항성과는 독립적이었으며 당뇨질환이 없는 폐경 후 여성을 대상으로 이루어졌다.

핵심되는 말 : 산화스트레스; 폐경; C-반응성 단백질; 산화된 저밀도 지단백질 (ox-LDL); 이소프로테인 (8-epi-$PGF_{2\alpha}$)